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Original Article

Echinococcus contamination ratio and its related risk factors in Moghan plain, northwest of Iran

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Abstract

Background and Objectives: Alveolar and cystic echinococcosis (AE and CE) are caused by the larval stages of *Echinococcus multilocularis* and *Echinococcus granulosus*, respectively. This study aimed to determine the prevalence of human AE and CE diseases among the tribes, livestock breeders, and farmers in Moghan plain, northwest of Iran.

Materials and Methods: Screening of hydatid cystic fluid antigen (HCF-Ag) was done by enzyme-linked immunosorbent (ELISA) kit. Briefly, HCF-Ag was collected, and the prevalence of the disease was evaluated using specific antigens for AE and CE (Ag-5, Ag-B, and $Em2^+$) and questionnaires. A total of 2453 serum samples were randomly collected from normal populations of five different areas of Moghan plain. The serology was evaluated using ELISA kit and specific antigens for AE and CE.

Results: Of the 2453 serum samples, 36 were positive for AE (1.46%). The infection rate was higher in males than females (1.94% vs. 0.94%). The age group of 4–19 years had the lowest and 40–59 years had the highest infection rate (0.2% vs. 2.5%). Among CE-positive serum samples, 178 samples were positive to Ag-5 (7.3%) and 167 samples were positive to Ag-B (6.8%).

Conclusion: Our findings showed that the highest infection rates were in the Borran and Eivazlou regions, and the lowest level of infection was for Parsabad area of Moghan plain. In areas with higher contamination, the risk factors associated with CE and AE were the water quality, the method of washing vegetables, and occupational and hydatid disease knowledge of the inhabitants.

Keywords: Ag-5, Ag-B, alveolar echinococcosis, cystic echinococcosis, $Em2^+$

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INTRODUCTION

Alveolar and cystic echinococcosis (AE and CE) are among the most serious chronic and zoonotic diseases in the world, causing major economic and health problems in the world. The causative agent is the family of human tapeworms with the genus and species of *Echinococcus multilocularis* and *Echinococcus granulosus* and definitive host of red fox and other canines (dogs). Various mammals including rodents (*Microtus socialis*), sheep, and humans are the intermediate hosts.[1] These global parasites are ranked as the second-most important medical diseases among worm infections.[2,3] The disease has been reported in eastern and southern Europe, the Mediterranean coast, the Middle East, Latin America, Africa, and Asia, being

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more prevalent in rural areas. Recently, new cases of *ortleppi* species of *Echinococcus* have been reported in East Asia and Vietnam, causing cyst in the lungs. From two cases of *Echinococcus ortleppi* infection, the first case had a cyst of 6 cm × 7 cm in diameter, and the second case had four cysts with diameters of 5 cm × 6 cm, 4 cm × 4 cm, 3.5 cm × 3 cm, and 2.5 cm × 2 cm, respectively. In both cases,[3,4] hydatid cysts (AE and CE) have been reported in various tissues including the liver, lung, and peritoneum, and can be found almost anywhere in the body. The main clinical symptoms of Echinococcosis are hepatomegaly, ascites, abdominal pain, splenomegaly, and lung and central nervous system disorders.[5] Both AE and CE have been reported in Iran, which accounts about 1% of the hospital surgery. The surgery rate increased recently due to the presence of Afghan refugees in Iran.[6] CE and AE are among the major public health threats, especially in rural and nomadic communities from different parts of Iran.[7,8] The prevalence of CE and AE has been reported in 2%–20% of intermediate hosts in different parts of the country.[1] The inhabitants of the Iranian countryside are mostly farmers, livestock, and shepherds. Therefore, AE and CE are considered to be a main risk to human health in countryside and can cause many economic and life losses.[8,9] However, literature survey showed that sero-epidemiological screening studies and the identification of risk factors associated with AE and CE disease are rare, and specially ultrasound diagnosis has not been performed accurately and completely along with serological studies. Considering the lack of the abovementioned information, the present sero-epidemiological and sonographic study was conducted to assess the prevalence and risk factors for AE and CE diseases among inhabitants of Moghan plain, Ardabil province.

MATERIALS AND METHODS

Statistical society

The present investigation is a cross-sectional and descriptive-analytic study. Informed consent of the participants was indicated in the written form, and the reasons and procedures used in the research were described to all the participants. Samples were collected from five major areas of Moghan plain as follows: area 1: Aslandouse (Ghareh-Ghabag, Torbat-Kandi, Hajiloo, Normohammad-Kandy, Kuroabaslou and related gullies, Eivazlou upper and lower, and Ghareh Tekanlou and related Giddies); area 2: Parsabad (Parsabad district, Islam abad, Oltan, Naderkandy, and Agh-ghabagh upper and lower); area 3: Borran (upper and lower, Khorooslou region, Ahmad Qeshlaghi, and areas overlooking the Giddies); area 4: Ider (Sefi Khanlou, Kechi Qeshlaghi, Gedailo, and Uvchi and related Giddies); and area 5: Bileh-Savar (Bileh-Savar, Jaafar abad, Shahre Babak, and Moghan agro-industrial zones and related Giddies areas). The total of 258,665 populations of Moghan plain is located in 630 km^2. According to the questionnaires, more than 80% of the households raise different domestic animals such as sheep, cows, camels, and goats. Mostly, spring and river water are used as water supplies for animals and drinking water. Analyzing the questionnaires determined that 74% of the population have a direct contact with dogs and consume wild vegetables including salsify, acarine, chamomile, and wild raspberry fruit without washing. Serums from households were tested by serologic methods (enzyme-linked immunosorbent [ELISA]), and those with clinical symptoms were introduced to the ultrasound center to perform abdominal imaging. The questionnaire had queries about the name, age, sex, occupation, owning dog, knowledge about AE and CE, literacy, consumption vegetables, the type of water used for drinking (plumbing or spring water and river), process of feeding the dog, dog housing, and history of hydatid cyst surgery.

Serologic method

Fifteen milliliters of blood was drawn from each of the volunteers and stored at −80°C until further serological studies. Commercial ELISA kits (Vircell, Granada, Spain) were used to evaluate the CE and AE antibodies in the sera. At first, hydatid cystic fluid antigen (HCF-Ag) screening was done, and then positive HCF-Ag was further investigated using specific antigens of B, 5, and Em2+. The collected data were analyzed using SPSS-21 software (SPSS Inc., Chicago, Illinois, USA). Relative risks were assessed by Chi-square and Fisher's exact tests. $P \leq 0.05$ was considered statistically significantly different. Cutoff point and related F value were detected using fifty healthy serum samples obtained from the Ardabil Blood Transfusion Organization. The mean optical density (OD) of the fifty healthy sera was detected three times, and the corresponding F value was calculated as follows:

F= 3D+average OD of fifty healthy Serum Samples/X-(3St -)

Then, cutoff points were considered for each microplate using the following formula:

Cutoff = F (multiplication factor) × X^{-} St^{-}.

Sonographic method

Abdominal sonography was performed on patients with symptoms to confirm the AE and CE infection. CE-type

hydatid cysts were classified in as simple, solid, and calcified. Patients with confirmed cystic images were referred for further serological study using Ag-5, Ag-B, and Em2+. Patients with positive serological tests who did not show any cyst in sonography were examined by chest computed tomography scan.

RESULTS

Statistical Society

A total of 2453 volunteers (1182 men and 1271 women) from five regions of Moghan plain (urban, rural, and nomadic populations) were enrolled in this study. The samples were divided into five age groups as follows: 4–19, 20–39, 40–59, 60–79, and over 80 years old [Table 1]. In the first step, for screening, all sera were tested using an ELISA kit and HCF-Ag coagulation tests. The logistic regression test was used to determine the odds ratio. In this test, the effect of different factors such as age, sex, owning the dog, consumption of wild vegetables, the method of washing and consuming edible vegetables, and the consumed water quality (plumbing, spring water, and river water) on serum results of ELISA test (positive and negative) was measured.

Serologic results

The present study showed that 37 out of the 2453 serum samples were AE positive (1.46%). AE infection was higher in men than women (2.03% vs. 0.94%). In the case of CE, 178 (7.3%) serum samples were positive for Ag-5 and 167 (6.8%) were positive for Ag-B-specific antigens, with a mean age of 47 years in the range of 6–81 years. CE infection was higher in women than men (8.2% vs. 6.26% for Ag-5 and 7.8% vs. 5.6% for Ag-B). The highest incidence of CE with Ag-5 and AE infection was seen in the age groups of 2 and 3, whereas the age groups of 1 and 4 had the lowest AE and the age groups of 1 and 5 had the lowest CE infection [Table 1]. In terms of occupation, the serologic results of the sample population showed that the highest CE contamination was in the field workers (9.6%) and the least contamination was in the student and teacher groups (4.6%). In the case of AE, the highest level of infection was in the farmer and livestock groups (1.7%), and the lowest level of pollution was in the homeworker group (0.2%). According to the sampling site, CE and AE serological results showed that the highest contamination was in area 3, i.e. Borran, Khorooslou, Ahmad Qeshlaghi, and destination areas for nomads. The lowest CE and AE infection rates belonged to districts 5 and 2, respectively. There was no significant difference between age groups, sex, and occupation, but a significant difference was seen between dog owners and place of dog keeping ($P < 0.004$ and $P = 0.000$, respectively). According to the place of dog keeping, side of flack had the highest rate on infection (12.3% and 3.2% for CE and AE, respectively).

Sonographic results

The results of sonography showed that 63 and 12 people had CE and AE, respectively. In CE-infected samples, cysts were seen in the liver (34 cases), Pulmonary organ (19 cases), abdomen (7 cases), subcutaneous tissue (2 cases), and brain (1 case) [Table 2]. Five samples were detected serologically positive while they did not show any cysts on sonography. Our survey indicated that they had a history of previous hydatid cyst removal operation. Nineteen individuals claimed that they had confirmed hydatid infection (4 AE and 15 CE), but in our ultrasound scan, they did not show any cysts. Therefore, taking both new and previous CE and AE cases into account, 6.7% and 1.4% (165 for CE and 33 for AE of the 2453 serums samples) of individuals of Moghan Plain were currently infected or have been infected with *E. granulosus* and *E. multilocularis*.

DISCUSSION

Comprehensive studies on AE and CE diseases, especially their risk factors, are very rare in Iran.[10] However, CE is more prevalent and has been reported from all countries in the Middle East and North Africa. Albeit *E. granulosus* is highly prevalent in Iran, Turkey, Iraq, Morocco, Tunisia, and Libya[1,2,11] AE also has been reported in these countries except Morocco and Libya.[5] CE is endemic in the Levant

Table 1: Frequency distribution of ELISA serologic test results for alveolar and cystic echinococcosis based on sex and age

Age group	Gender		Number of positive cases (%)						Total	
			Men			Women				
			CE		AE (Em2+)	CE		AE (Em2+)	CE	
	Men	Women	Ag5	AgB		Ag5	AgB		Ag5	AgB
19-4	269	286	12 (4.56)	11 (4.1)	1 (0.38)	15 (5.1)	14 (4.8)	0 (0)	27 (4.8)	25 (4.5)
39-20	327	369	21 (6.6)	20 (6.2)	8 (2.5)	30 (8.02)	28 (7.4)	4 (1.06)	51 (7.3)	48 (6.9)
59-40	269	296	24 (9)	22 (8.2)	9 (3.3)	37 (12.2)	36 (11.9)	5 (1.6)	71 (10.6)	58 (10.2)
79-60	228	229	13 (5.8)	12 (5.3)	4 (1.7)	18 (7.2)	17 (7.2)	2 (0.85)	31 (5.3)	29 (6.3)
≥80	89	91	4 (4.7)	2 (2.3)	2 (2.2)	5 (5.4)	5 (5.1)	1 (1.02)	9 (4.9)	7 (308)
Total	1182	1271	74 (6.26)	67 (5.6)	23 (1.94)	104 (8.2)	100 (7.8)	12 (0.94)	178 (7.3)	167 (6.8)

CE: Cystic echinococcosis, AE: Alveolar echinococcosis

countries, Cyprus, and Oman.[1] Various surveys have found hydatid cysts in sheep, cattle, goats, and camels through these regions.[2,5,6] There is no study on the AE and CE risk factors in the Moghan region. The present study was carried out to determine echinococcosis using serological and sonological methods, as well as to investigate the prevalence and related risk factors. Contamination with CE and AE indicates a serious public health threat in Iran and Moghan plain in the northwest of Iran.[8] The high contaminations in the age groups of 20–39 and 40–59 [Table 1] are probably due to occupation and high contact with dogs and the surroundings.[10,12-13] A similar study in Jordan showed the highest rate of infection among children. Furthermore, the highest prevalence among children aged 16 years has been reported from Kyrgyzstan.[14] The controversy of our results and those reported from other countries may rise from lifestyle differences between the nations. Our data showed that the infection rate is higher in the Borran and Eivazlou areas in comparison with Parsabad district (Nadarkandi and Agh-Ghabagh). The authors suggest that the low contamination in Parsabad district is related to the available refined tap water in this region. In this study, the rate of infection was low among those who were washing vegetables with detergents ($P = 0.003$ and 0.004 for CE and AE, respectively), and therefore one of the risk factors for hydatid cysts would be the situation of vegetable consumption. According to the questionnaire, those who had more knowledge about AE and CE had less infection. On the other hand, the infection rate was higher in women than men, which is in consistent with the reports from the Middle East and North Africa.[15] The increased exposure of women to infectious agents such as vegetables may explain their highest risk albeit other studies which claimed that it is likely due to higher treatment-seeking behavior in women. In terms of occupation, the highest and least CE and AE contaminations were seen in field workers and students, respectively, although their differences were not significant.

Table 2: Distribution of ELISA serological results for alveolar and cystic echinococcosis based on the sampling site

Sampling site	Regions	Percentage of positive cases (%)				Total
		CE	AE	CE	AE	CE
Area 1		5.1	0.42	94.9	99.58	100
Area 2		6.3	0.3	93.7	99.7	100
Area 3		12.1	2.1	87.9	97.9	100
Area 4		9.2	1.3	90.8	98.7	100
Area 5		3.8	0.7	96.2	99.3	100

CE: Cystic echinococcosis, AE: Alveolar echinococcosis

CE and AE were higher in spring water consumers than those who use tap water. Contaminated dogs are direct or indirect sources of CE infection for humans, and therefore the prevalence of infection and contamination of dogs are among the most reliable indicators and risk factors for human infection. The prevalence and distribution of *E. granulosus* in herds of dogs have been investigated in different provinces of Iran, but there has been no accurate and complete study on the infection rates in the hosts of *E. multilocularis*. In Markazi and Esfahan provinces, adult *E. granulosus* worms have been found in 27.17% of dogs.[16,17] In a diagnostic study, the sampling sites showed that most of the dog keepers' residents are infected, which significantly relates to the place that Dogs has been hosted.

The results of the logistic regression analysis are presented in Table 3. Contamination based on the occupation and the

Table 3: The results of logistic regression analysis and odds ratio for cystic echinococcosis based on gender, occupation, location of sampling, and method of washing vegetables

Risk factor	*n*	Raw logistic regression		*P*	Adapted logistic regression, Odds ratio (CI)
		Prevalence (CI)	Odds ratio (CI)		
Sex					
Male	1182	4.2% (4.92-3.65)	1	1.54	1
Female	1271	6.4% (7.94-4.56)	1.303 (84/1-93/0)		1.54 (283/2-038/1)
Occupation					
Agriculture-animal husbandry	854	6.2% (5.4-8.91)	1	-	1
Free job	545	9.6% (5.91-11.3)	1.115 (95/1-64/0)	0.703	1.63 (040/3-871/0)
Homemaker	626	5.2% (79-40/6)	0.852 (20/1-60/0)	0.355	0.774 (171/1-511/0)
Student and teacher	428	4.6% (82/9-38/3)	0.633 (112/1-36/0)	0.112	0.957 (751/1-523/0)
Area					
Area 1	561	5.1% (4.90-6.24)	1	-	1
Area 2	450	6.3% (3.62-6.33)	1.178 (5/2-55/0)	0.670	1.166 (506/2-541/0)
Area 3	542	12.1% (4/10-13.3)	1.7.6 (94/2-99/0)	0.054	2.119 (846/3-169/1)
Area 4	447	9.2% (7.32-9.64)	2.342 (69/4-17/1)	0.017	2.457 (155/5-71/1)
Area 5	453	3.8% (2.6-5.1)	1.163 (97/1-69/0)	0.574	1.242 (207/2-699/0)
How to wash vegetables					
Washing with dishwashing liquid	795	4.2% (4.88-6.11)	1	-	1
Washing with salt water	812	3.6% (2.24-4.36)	1.715 (0.911-2.69)	0.018	1.49 (0.917-2.41)
Washing only with water	846	6.8% (7.31-8.12)	1.715 (1.092-2.46)	0.017	0.91 (0.569-1.462)

CI: Confidence interval

manner of washing vegetables is not significant, but it is significant regarding the sex and sampling areas [Table 3]. In countries with endemic AE and CE, any growing mass must be suspected to hydatid disease.[18] In the present study, cysts were found in 73.8% of the cases in the liver and 17.7% in the lung, whereas they were also found in the spleen (2.5%), abdomen (1%), brain (1.5%), both liver and lung (1%), and other organs (2.5%). Therefore, liver and lungs account for >90% (91.5%) of cysts according to reports of Sadjjadi *et al*.[1] and Azordegan *et al*.[19,20] Considering the outstanding clinical resemblance between hydatid disease and malignant diseases in some organs, it is essential to diagnose the disease correctly. The findings of this study provide a useful tool for identifying the diagnosis, prevalence, and risk factors of both cystic and alveolar hydatid diseases. Serological method is necessary for following the patients treated with the surgery. Although we had some limitations in our investigation such as migration of nomad tribes, people refrain from entering the study, and access to the rural area, we did our best to overcome these problems.

CONCLUSION

We aimed, study accurately assess the ratio of echinococcosis (AE and CE) infection and related risk factors in Moghan Plain.

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Conflicts of interest
There are no conflicts of interest.

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